IN VITRO CONTRACTILITY OF ATRIAL MYOCARDIUM FROM HYPERTHYROID GUINEA PIGS

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by

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TABLE OF CONTENTS

INTRODUCTION

Myocardial norepinephrine (NE) concentration is increased in the hyperthyroid animal (6). The increase in cardiac output and heart rate seen in hyperthyroid animals may be caused in part by this increased NE content of the myocardium in response to thyroid hormone (6).

The addition of NE to the bath of beating heart muscle preparations *in vitro* will increase the strength of contraction (7, 23, 24, 25, 26, 27). Decreasing the endogenous stores of NE in heart muscle of animals prior to *in vitro* studies by means of reserpine or sympathectomy results in a decrease in the strength of contraction. (1)

Investigations by Benforado (2, 3), Whitehorn and Ullrick (4), and Brewster (5), have shown that the myocardium of hyperthyroid animals develops decreased isometric tensions at given resting loads and frequencies compared to normals, while hypothyroid myocardium develops greater tensions. This finding does not seem to be in keeping with the established findings of an increase in cardiac output and endogenous catecholamine content of the hyperthyroid animal.

Although numerous studies have been done on the mechanism of heart muscle contractility and its responses to many varied

mechanical, electrical, drug, and hormonal influences, very few studies have been made of the influence of the hyperthyroid and hypothyroid state on myocardial contractility. Atrial and ventricular muscle strips from hyperthyroid rats developed less mean isometric tension than enthyroid controls studied at similar resting tensions and frequencies (3). On the other hand, hypothyroid rats developed greater isometric tensions than controls (2, 3). The author suggested that these results were compatible with an uncoupling of oxidation from phosphorylation by thyroid hormone.

Whitehorn and Ullrick (4) compared the isometric contractions of ventricular muscle strips from hyperthyroid and normal rats and found that hyperthyroid myocardium developed significantly less tension per milligram of muscle weight than control strips at all resting tensions and initial lengths. In addition, the mean maximal developed tension of the control group was significantly greater than that of the hyperthyroid rats.

An inverse exponential relationship was observed to exist between the metabolic rate and the duration of the isometrically contracted state or time required for relaxation of ventricular myocardium in vivo in athyroid, enthyroid, and hyperthyroid

dogs (5). Specifically, as a consequence of the decreased metabolic rate in athyroid animals, the duration of the contracted state was prolonged, thus producing a greater total strength of contraction than that produced by hearts of normal or hyperthyroid animals under similar conditions. The authors thus demonstrated that the average total myocardial contractile force developed in vivo in hypothyroid dogs was greater than in controls, while that developed in hyperthyroid animals was less than in controls, over a temperature range from 27 to 37° C.

The results of all these studies have indicated that hyperthyroid myocardium develops less isometric tension than controls under similar experimental conditions. No attempt was made in any of these studies to measure the myocardial NE content. Attempts to correlate the myocardial NE content with contractility have involved the study of the effects of drugs which stimulate autonomic nerves and the effects of depletion of NE stores by reserpine or sympathectomy. Lee and Shideman (1, 8) measured the contractility of cat papillary muscle in vitro in animals depleted of catecholamines by either reserpine or bilateral sympathectomy and found decreased myocardial contractility in the treated animals as compared to the normals. In another experiment, subsequent administration of NE or epinephrine to

the NE depleted papillary muscle resulted in a marked positive inotrophic response. The authors interpreted these results as demonstrating the importance of normal levels of myocardial catecholamines in the maintenance of normal cardiac contractility.

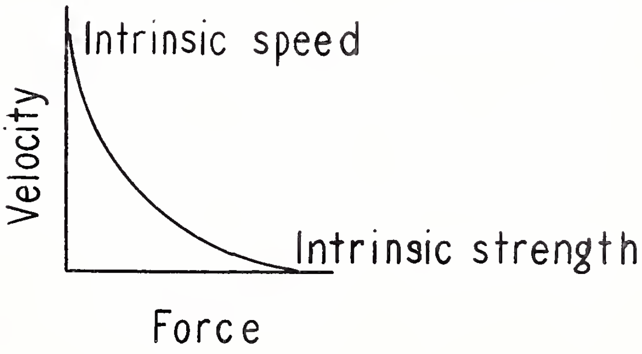
Previous reports have demonstrated that the release of NE from stores within the myocardium by means of the administration of certain sympathomimetic drugs is responsible for the increase in myocardial contractility resulting from these drugs (8, 9, 10, 11). Evidence indicates that the parasympathomimetic and sympathomimetic effects of ganglionic stimulating substances arise from the liberation of acetylcholine and norepinephrine respectively (8, 9, 11, 28, 29, 30). For example, reserpine abolishes the positive inotrophic response to nicotine usually seen in the atropinized myocardium, thus indicating the important role NE can play in the positive inotrophic effect of certain drugs (10).

The purpose of the present study is to compare the myocardium of hyperthyroid and enthyroid animals to determine whether heart muscle from the hyperthyroid animal studied in vitro has (1) an increased NE content and (2) a decreased isometric tension compared to normals.

DEFINITION OF TERMS

In order that the meaning and significance of terms used in the present study are fully understood, certain definitions are essential. Since the term myocardial contractility will be used in reference to (1) the specific property of heart muscle and (2) a specific numerical index with which to compare performances of different heart muscles, it requires defining.

A. V. Hill and collaborators have defined muscle contractility in terms of fundamental muscle mechanics (15, 16). They describe a contracting muscle as composed of three elements. The first is an active contractile component made up of two independent properties; the capacity to shorten and the ability to develop tension. When considered in relation to one another, the two variables determine a force-velocity relationship. The latter is a consequence of the observation that the velocity of shortening of the contractile component is uniquely determined by the tension in the muscle; the greater the tension, the lower the velocity of shortening. A plot of these two interrelated variables appears below as a curved line, convex toward the origin, intersecting the ordinates at finite values of force and velocity.



The intrinsic speed, termed Vmax by Sonnenblick, is the maximum speed at which a muscle shortens under zero load. The intrinsic strength is the maximum tension exerted per unit cross sectional area when the muscle is not permitted to shorten. Sonnenblick found that increasing the fiber length of heart muscle would increase the intrinsic strength but not Vmax. On the other hand, he found that "inotrophic interventions", i.e. those interventions which increased the strength of contraction exclusive of altering the fiber length, involved a change in Vmax. The conclusion was that Vmax helped to define and quantify the basic state of heart muscle, i.e. its contractility (7).

The other two theorized components of muscle are two passive elastic components, one mechanically in series and the other mechanically in parallel with the contractile component. These two components are not involved in the actual contractile process, but their presence modifies the behavior of the muscle. The most important modification is due to the presence of a relatively inextensible parallel elastic element. Due to its lack of extensibility, the parallel elastic element is responsible for bearing the full resting tension of the heart muscle. This inextensibility of the parallel elastic component is a distinguishing feature of heart muscle, for skeletal muscle lacks this feature

and thus does not develop resting tensions. In the process of shortening of heart muscle during contraction, however, progressively less tension is born by the parallel elastic component, thus resulting in a transfer of part of the resting tension to the contractile element. As a consequence of this phenomenon, at high levels of resting tension the difference between the resting tension and peak systolic tension developed in an isometric contraction may be an inaccurate measure of the tension actually developed by the contractile component. This phenomenon might also be responsible for the insensitivity of isolated heart muscle preparations to interventions which alter strength of contraction when the latter is low compared to the resting tension (12).

Finally, a muscle may be considered to be in an "active state" when the contractile component is actively shortening or exerting force (12).

Koch-Weser and Blinks (12) define a change in myocardial contractility as occurring when the strength of contraction is altered by changes in (1) the degree of activation, and (2) the duration of the active state. The authors redefine these two theoretical conditions in terms of familiar measurements used in the study of heart muscle behavior. First, a change in the degree of activation occurs if there has been a change in the

rate of shortening or the development of tension in contractions recorded under similar conditions. In particular, an increase in the degree of activation will be reflected in an increase in peak tension developed in an isometric contraction. Second, a change in the duration of the active state occurs with any change in time from the first detectable development of tension to the maximum tension of an isometric contraction (time to peak tension). Increases in the time to peak tension reflect increases in the duration of the active state.

It is interesting to note in light of the above definitions that changes in the tension developed in heart muscle as a consequence of changes in the fiber length do not represent changes in myocardial contractility, since the activity of the muscle is unaltered (12, 13). As a corollary to this, Sonnenblick noted that increasing the fiber length did not change the time from the onset of contraction to peak tension if all other factors were constant (7).

Sonnenblick proposes one other means of assessing and measuring a change in myocardial contractility. He observed that the ratio of the rate of tension developed (dp/dt) to the integrated developed isometric tension (iit) was a constant for any one state of contractility independent of muscle length both <u>in vivo</u> and <u>in vitro</u> (14). Thus a change in the rate of tension

developed (dp/dt) alters this ratio and also reflects a change in the degree of activation, both effects being equivalent to a change in myocardial contractility.

In the present study, isometric contractions are used as the index of behavior of the heart muscle preparation in vitro under certain imposed experimental conditions. Changes in the behavior of the heart muscle consequent to the altered physiological state in the hyperthyroid guinea pig will be sought in possible changes in myocardial contractility. In light of the definition of such changes in myocardial contractility presented above, three variables will be studied:

(1) The ratio of dp/dt to developed tension, a constant for any given muscle independent of fiber length if no inotrophic interventions occur.

(2) The time to peak tension.

(3) The peak tension developed.

Since precise comparisons of peak tension developed between two heart muscles requires measurements at comparible resting tension, variables not measured in the present study, only rough comparisons are possible. Whitehorn and Ullrick's method is used in which comparisons of the maximal tension developed per unit weight of muscle were made between heart

muscle at similar initial lengths.

Finally, factors known to influence myocardial contractility, such as temperature and frequency of stimulation, must be taken into account when comparisons are made between contractilities of different heart muscle preparations.

MATERIALS AND METHODS

1. Materials

A special muscle chamber (2) was suspended in a large volume water bath (1) kept at a relatively constant temperature. As diagrammed in Figure 1, the chamber consisted of a glass cylinder 3 x 15 cm. in size at the bottom of which were placed two inlets, one for admitting gas and the other for changing the muscle bath solution. A gas mixture was bubbled through the central inlet (3) to oxygenate the Chenowith-Koelle (C-K) solution (see Appendix I) in the muscle chamber.

The C-K solution could be changed at intervals by means of Y-tubing (6) connected to the side inlet (5), and the muscle chamber thus refilled with fresh solution from the storage bottle(7).

The apparatus for support and stimulation of the muscle was suspended in the muscle chamber. This consisted of a glass tube (8) in which was housed two stimulating electrodes, and which had a hook at its lower end to serve as an attachment site for one end of the muscle. The stimulating electrodes projected through the wall of the glass tube into the solution and were placed near the surface of the muscle. The heart muscle was tied at its lower end to the glass hook and at its upper end to an

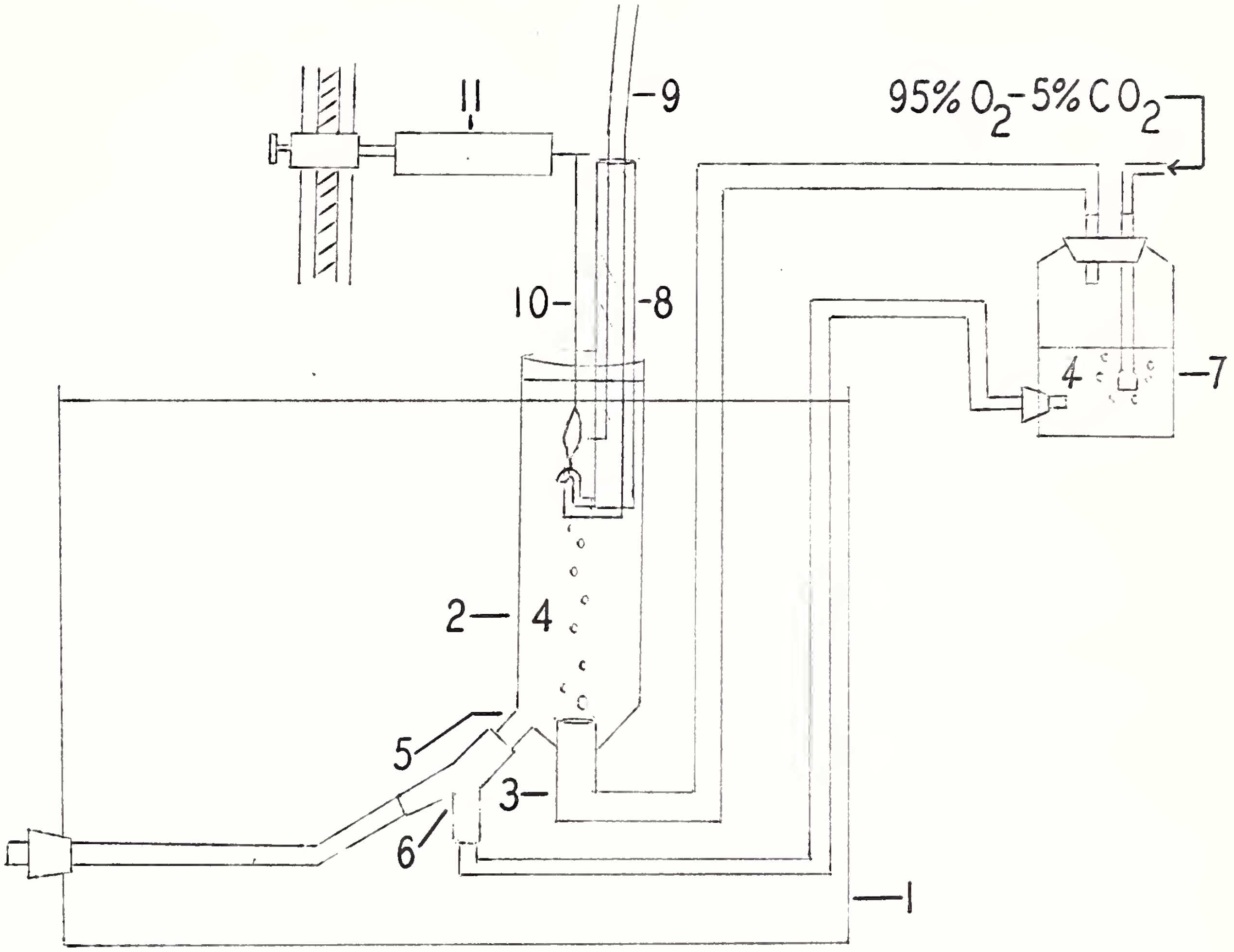


Diagram of Apparatus

Figure 1

inextensible metal chain (10), which was attached in turn to a Statham strain gauge (11) for recording of isometric contractions. The strain gauge was attached to a support in such a way that its height could be varied, thus in turn varying the length and resting tension of the muscle (12).

The recording apparatus consisted of (1) the Statham strain gauge (2) a D-C amplifier and (3) an oscilloscope and photographic recorder (Electronics for Medicine). The muscle contractions and the first differential of the muscle contractions, i.e. the rate of change of developed tension per unit time (dp/dt), were recorded simultaneously on the oscilloscope screen. Representative tracings were recorded on photographic paper for later analysis of data.

An American Electronics Laboratory square wave stimulator model #104 A was used to drive the muscle.

2. Methods

Guinea pigs were used as the experimental animal. Male guinea pigs weighing between 200-500 grams were selected, and a group was made hyperthyroid by the intraperitoneal injection of 100 g of L-thyroxin per day for a period of 7-14 days.

The animals were sacrificed, the heart was removed,

and right and left atria were dissected free while the heart was being bathed in oxygenated C-K solution. Small sized skin clips (7 mm.) were applied to the ends of each atrium for attachment in the muscle bath. As a result of the firm fixation of the muscle, no shortening was possible, and isometric contractions were recorded.

Stimulation of the muscle was begun at low frequencies, approximately 1.5 beats per second, with a constant supravoltage value of 10 volts and a duration of stimulation of 1 millisecond. The point of zero resting and developed tension was then found by varying the length of the muscle until a just noticeable positive upward deflection of contractile force was observed above the zero resting tension, beyond which a small increase in length of 0.5 mm. would significantly increase the positive deflection. The length of the muscle at this zero resting tension setting was called the initial length, and this was measured with a pair of calipers as the length of muscle between the skin clips. All changes in length were then expressed in the data as a percentage increase in length above the initial length, in order to compare similar values between atria.

The bath temperature varied from one experiment to another, the range for the whole study being between 21°C and

28°C. For each experiment, however, bath temperature remained relatively constant, varying not more than 1°C over the course of the experiment.

The experimental procedures used over the period of the present study were divided into two categories according to the methods of studying the behavior of the left atria. The procedures used for the right atria were standardized throughout the course of study. The two categories of experimental procedures for study of left atrial contractility are summarized below.

Category I.

Left atria were mounted and adjusted to zero resting and developed tension as described above. The atria were then stimulated at a constant frequency chosen in most instances from the frequency giving the maximal contractile force in the right atrium of the same animal, the procedure of which is described below. When left atria were placed in the bath first, however, this frequency was chosen from the frequency at which maximal contractile force was obtained when frequency was varied at a low length setting. This frequency so obtained was essentially the same for all length settings.

The muscle length was slowly increased in increments

of 0.5 mm and contractile response recorded for each length setting at a paper speed of 10 cm/sec and 200 cm/sec, with time line markings of 1.0 sec and 0.02 seconds respectively. For each atrium developed tension ceased to increase with each increment increase in length at a muscle length 5-8 mm above the initial length. When developed tension remained constant or decreased with an increase in length, recordings were stopped. A length-tension curve was thus obtained at a single frequency of stimulation.

Category II.

Left atria were mounted and adjusted as before. The heart muscles were stimulated at varying frequencies for each increment in length. Frequencies were varied between 1.5 and 3.5 beats per second. In this interval, a frequency was found which produced a maximal contractile response (optimal frequency). Procedures were otherwise the same as in category I. A series of length-tension curves were thus obtained, one curve for each frequency of stimulation used.

The experimental procedures used on the right atria were the same as those used in category II. Comparisons between right and left atria of the same animal were thus possible as well as comparisons between atria of different animals.

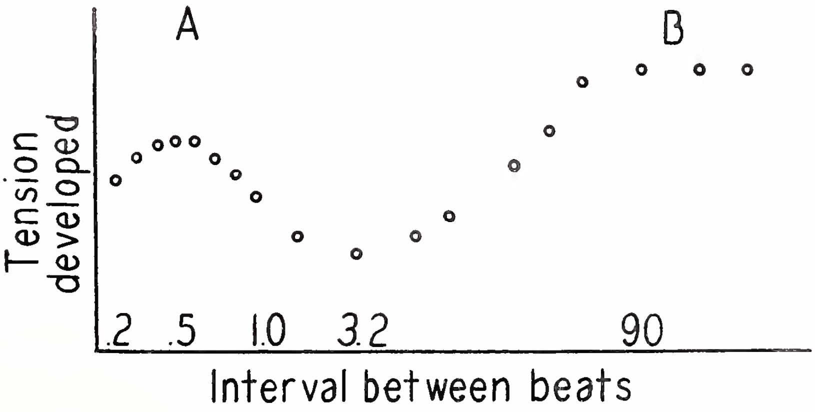
At the end of each procedure, the individual atrium was weighed and placed in a 0.4% perchloric acid solution for homogenization and analysis of norepinephrine content according to a modification of the method of Bertler et al. (17). The length of time the atrium had been in the muscle bath was also noted.

RESULTS

A. General Results

Figure 1 shows the average length-tension responses of atria from groups of normal and hyperthyroid animals. Developed tension for both groups of animals rose gradually with increasing length followed by a gradual fall as the muscle was progressively lengthened beyond approximately a 90% increase over the initial length. The shape of these curves is in agreement with innumerable studies of the isometric tension developed by cardiac muscle in response to changes in length. The variance between the curves of the hyperthyroid and control animals will be discussed in the next section.

The biphasic curve shown in Figure 2 is in general agreement with the studies of Koch-Weser and Blinks on the effect of the interval between beats on myocardial contractility (31). A plot of the interval-strength relationship of mammalian atria in their studies showed a triphasic curve as illustrated below:



The optimal frequency was termed that frequency in the curve labelled A at which strength of contraction was greatest. The frequencies employed in the present study ranged between 1.5 beats/second and 3.0 beats/second, which corresponds to an interval between beats of .67 seconds to .33 seconds respectively. Thus, the biphasic curve obtained in the present study corresponded closely to curve A in the Koch-Weser and Blinks study.

Finally, Figure 3 demonstrated the linear relationship between developed tension and the maximal rate of development of tension (dp/dt). This finding was in agreement with Sonnenblick's proposal that the ratio of dp/dt to the integrated developed isometric force was a constant for any one state of contractility independent of muscle length.

B. Comparison of Results between Normal and Hyperthyroid Animals

I. Graph of dp/dt to developed tension.

Figure 3 demonstrates the linear relationship of the ratio of dp/dt to developed tension. The average ratio of dp/dt to developed tension for the hyperthyroid group was .94, while that for the controls was .77, significantly different from one another at the $P < .001$ level. Due to the two different experimental procedures followed using the left atria, as outlined in the

preceding section, a statistical analysis was done which showed that there was no significant difference between the results obtained by these two procedures ($P > .05$). The results of the two different procedures were therefore pooled, and the final statistical analysis was preformed by matching a normal atrium with a hyperthyroid atrium for which frequency of stimulation and bath temperature were comparible, thus minimizing variations in myocardial contractility due to these factors (see Appendix II). Figures 4 and 5 demonstrate the definite grouping of hyperthyroid atria above the controls when these factors were accounted for.

Similar results for the relationship of dp/dt to developed tension between hyperthyroid and control groups were obtained for the right atria. The average ratio of dp/dt to developed tension for the hyperthyroid group was .89, while that for the controls was .71, significantly different from one another at the $P < .001$ level. The variation of the ratio of dp/dt to developed tension with bath temperature and frequency of stimulation for the right atria of the two groups was similar to the variation of these factors seen with the left atria.

a) dp/dt value at a given tension of 500 milligrams on dp/dt - developed tension curve.

Comparison of dp/dt values at a given tension of 500 mg. between the two groups of animals showed a significant difference ($P < .003$), with an average dp/dt value of 10.15 mg/ms for the hyperthyroid group as compared to 8.10 mg/ms for the controls. Variations due to bath temperature and frequency of stimulation were minimized by matching atria as before. Results for the right atria were also significant ($P < .002$), with an average dp/dt value for the hyperthyroid group of 8.74 mg/ms compared to 7.04 mg/ms for the control group.

II. Time to Peak Tension

Hyperthyroid atria had a significantly shorter time to peak tension (7.89 milliseconds) than enthyroid atria (9.48 milliseconds), ($P < .002$). The time to peak tension was found to be constant at any given frequency over the range of resting tensions and length settings employed for each atrium. The time was obtained from the recording strip run at a speed of 200 cm/sec.

Figures 6 and 7 demonstrate the grouping of hyperthyroid atria at a lower time to peak tension than controls when both frequency of stimulation and bath temperature were accounted for as before. In addition, these figures suggest a decrease

in the time to peak tension with increases in both frequency of stimulation and bath temperature.

Finally, Figure 8 demonstrates the observation that the greater the ratio of dp/dt to developed tension, the shorter the time to peak tension. Hyperthyroid animals were thus grouped at the higher levels of dp/dt and lower times to peak tension.

III. Ratio of Maximal Tension developed to the Weight of the Muscle (Tmax/mg muscle)

Comparison of the amount of tension developed per milligram of muscle between right and left atria of the same animal showed that both atria developed approximately the same Tmax/mg muscle. There was no significance however between the Tmax/mg muscle values of hyperthyroid and normal left atria ($P > .05$). Attempts to match atria according to comparible frequencies of stimulation and bath temperature yielded only 4 such pairs. Comparisons among this small group, however, shoed no definite difference in Tmax/mg muscle between hyperthyroid and control animals.

Graphs of Tmax/mg muscle as (1) frequency of stimulation and (2) bath temperature did not show the separation of hyperthyroid animals from controls as had been observed in Figures 4 - 7.

IV. Tension Developed at a 50% Change in Length or Length-tension Curve.

No significant difference $P > .05$ was found between the tensions developed per milligram of muscle at a 50% change in length in the left atria of the two groups of animals. In addition, no significant difference was found in the percentage change in length at which peak developed tensions were observed.

V. Average Frequency of Stimulation at which Maximal Developed tensions Obtained (optimal frequency)

Hyperthyroid right atria were found to have a significantly higher optimal frequency (mean 163 beats/min.) than controls (mean 136 beats/min) ($P = .046$). Atria were paired between the two groups according to comparable bath temperatures in order to minimize errors in the analysis.

Figure 9 shows the grouping of hyperthyroid animals at a slightly higher optimal frequency for any given bath temperature than controls. It was noted that the optimal frequency decreased with decreasing temperature, in agreement with Kruta's work (18-20).

VI. Relation Between Myocardial NE Content and Contractility.

No correlation was found between the NE content of the

atrium and its contractility ($P > .05$). In addition, there was no significant difference in the myocardial NE content between hyperthyroid and normal guinea pigs.

Comparison of NE content between right and left atria of the same guinea pig showed the right atria had a consistently higher NE content than the left. In the hyperthyroid group, the mean for the right atria was 3.73 μg/g and for the left atria 3.03 μg/g. There was no significant difference between these two values however, among 15 hyperthyroid animals. In the 6 normal animals studied, the mean for the right atria was 4.43 μg/g while that for the left was 2.63 μg/g, significantly different from one another at the $P > .01$ level.

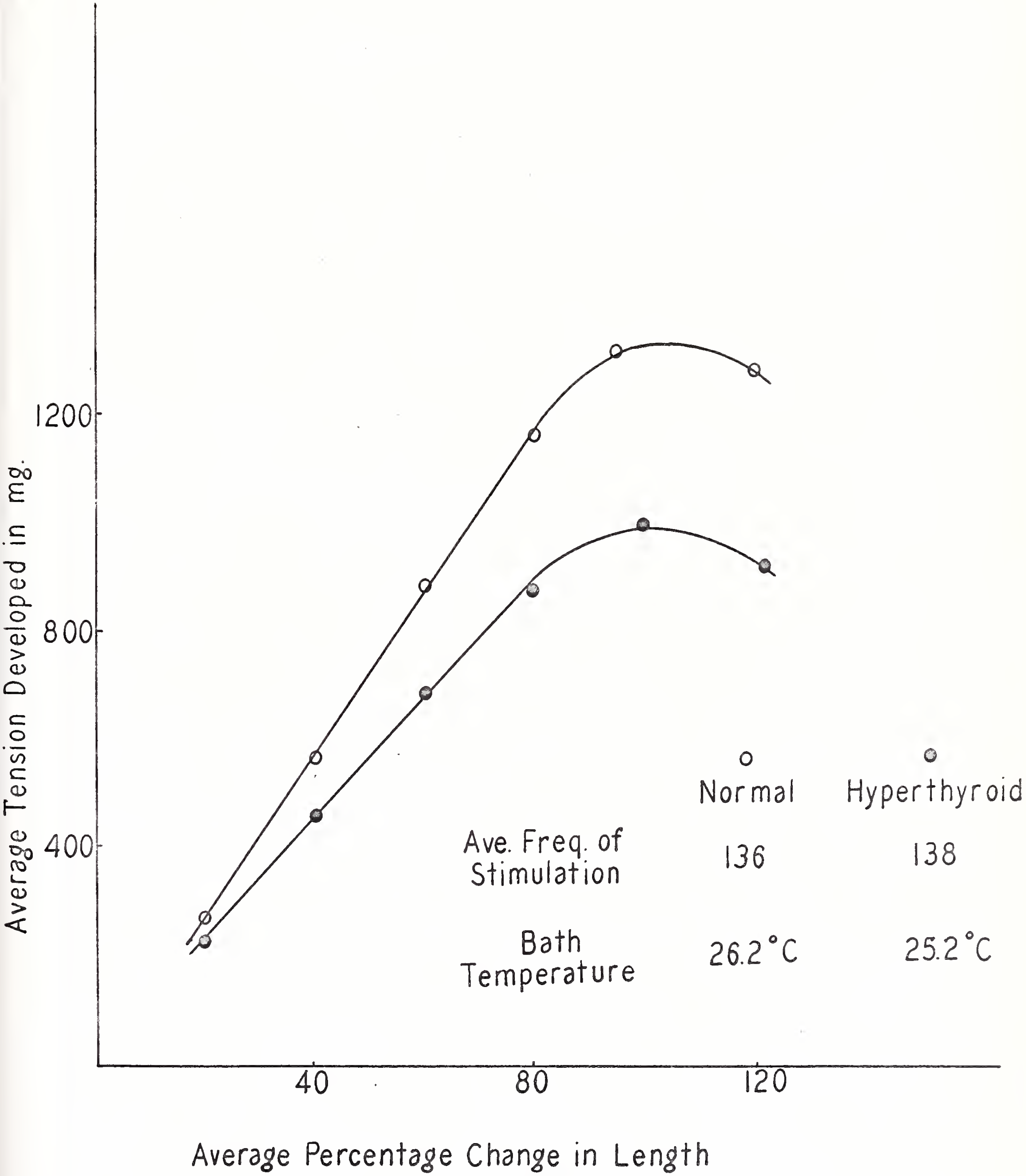

Average Tension Developed in mg.
1200
800
400
40
80
120
Average Percentage Change in Length
○ Normal
● Hyperthyroid
Ave. Freq. of Stimulation
136
138
Bath Temperature
26.2 °C
25.2 °C

Figure 1

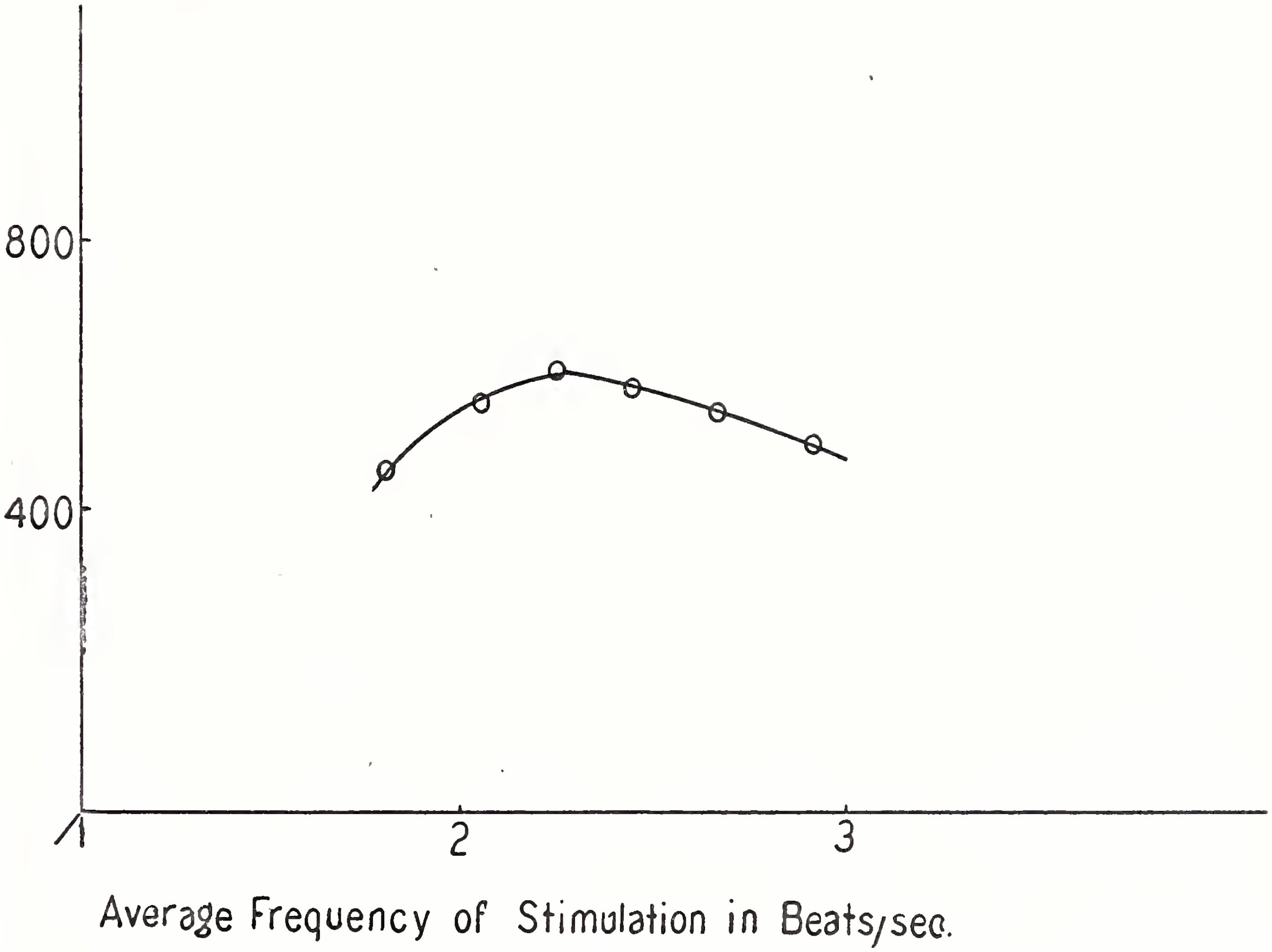

Tension Developed in mg.
800
400
1
2
3
Average Frequency of Stimulation in Beats/sec.

Figure 2

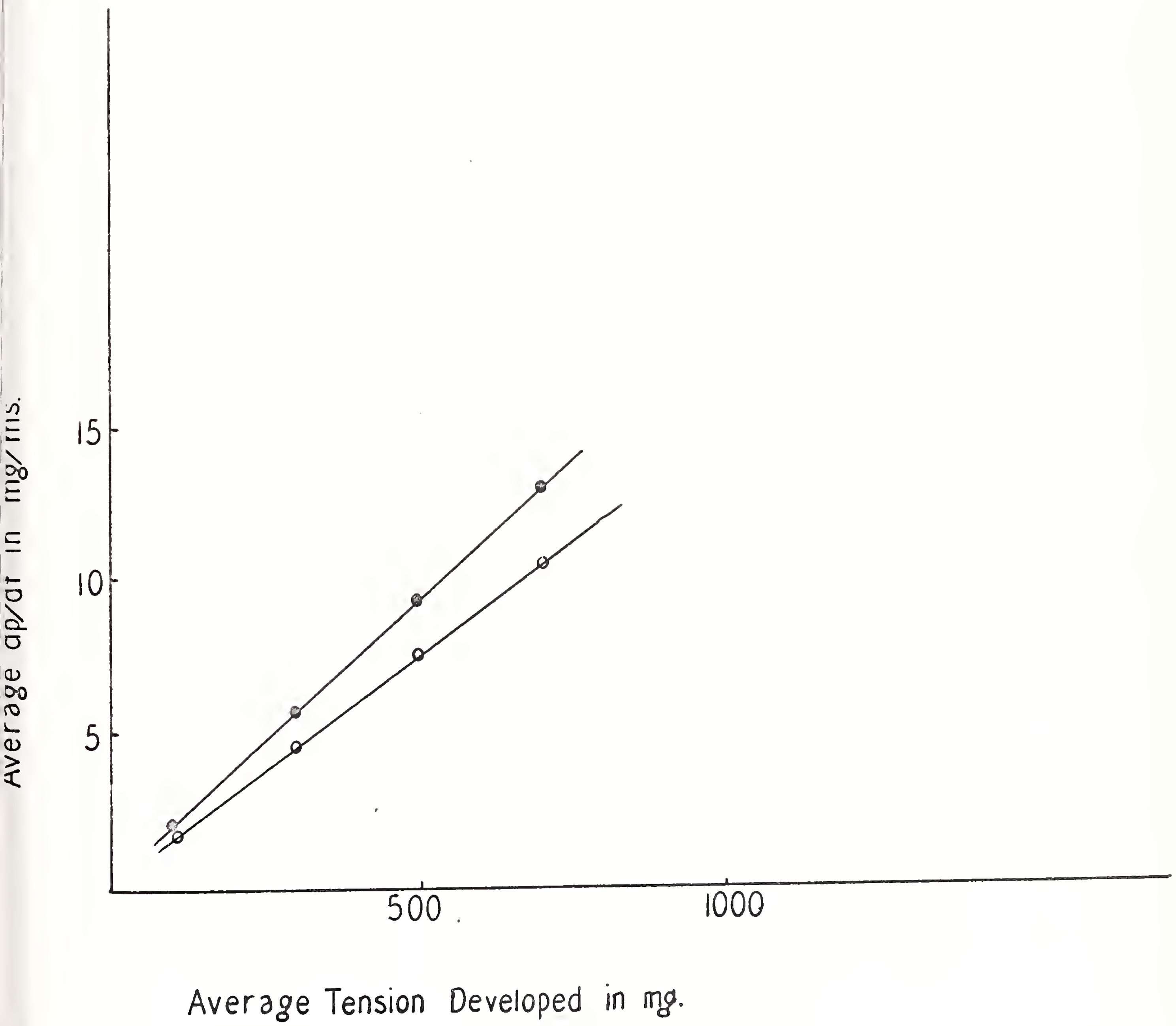

Average dp/dt in mg/ms.
15
10
5
500
1000
Average Tension Developed in mg.

Figure 3

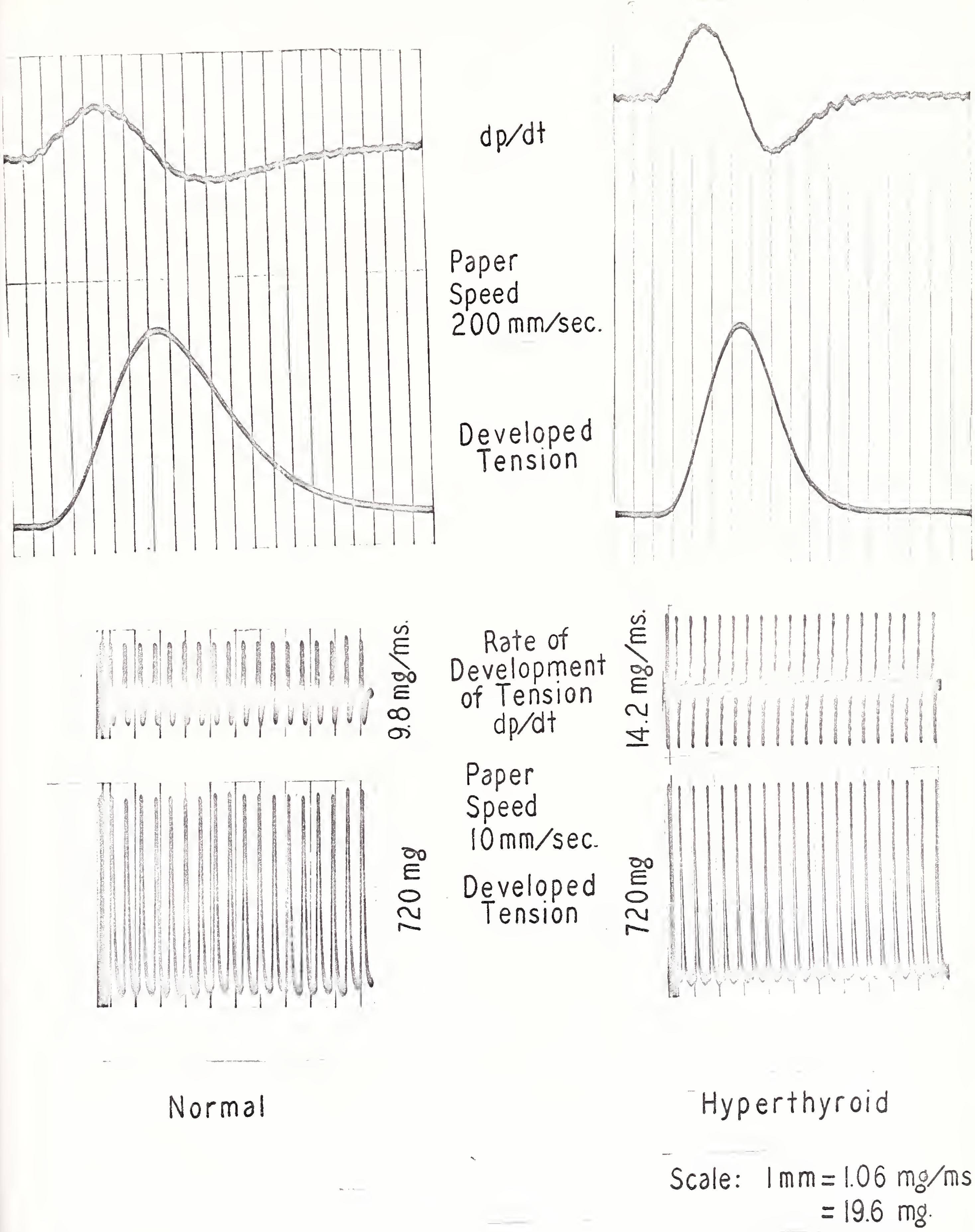

dp/dt
Paper Speed 200 mm/sec.
Developed Tension
9.8 mg/ms.
Rate of Development of Tension dp/dt
14.2 mg/ms.
Paper Speed 10mm/sec.
720 mg
Developed Tension
720mg
Normal
Hyperthyroid
Scale: 1 mm = 1.06 mg/ms
= 19.6 mg.

Figure 3a

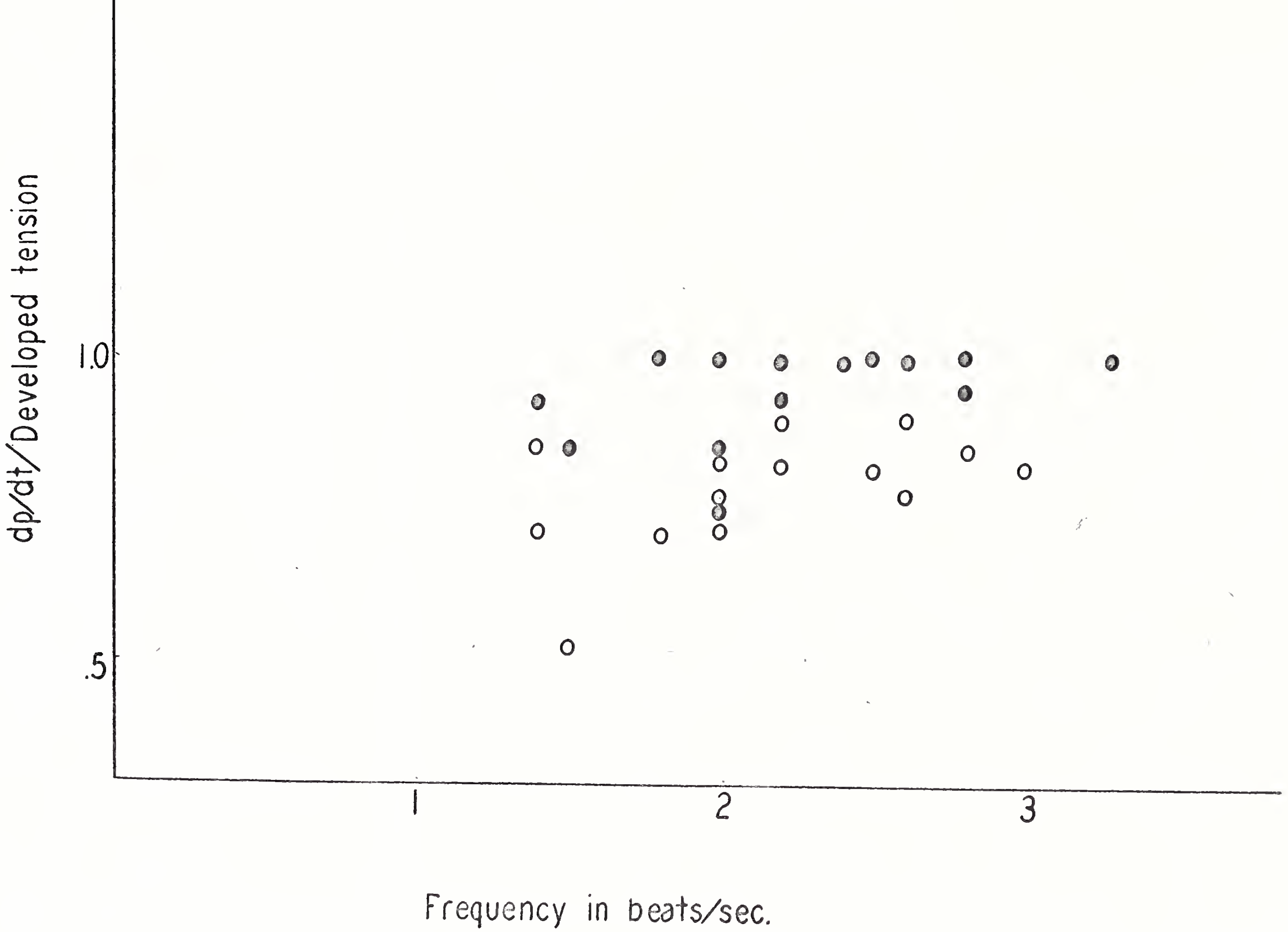


Figure 4

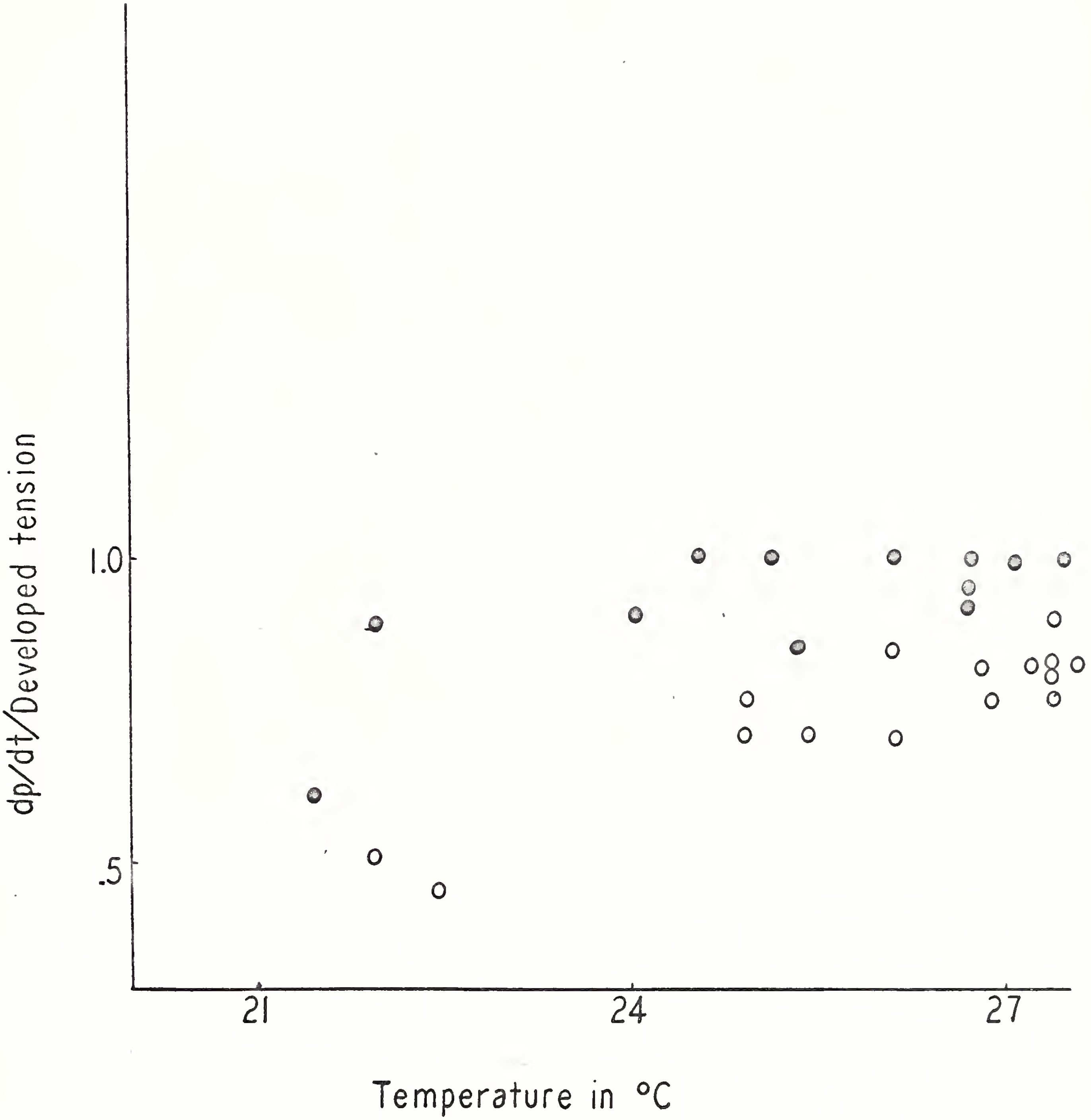


Figure 5

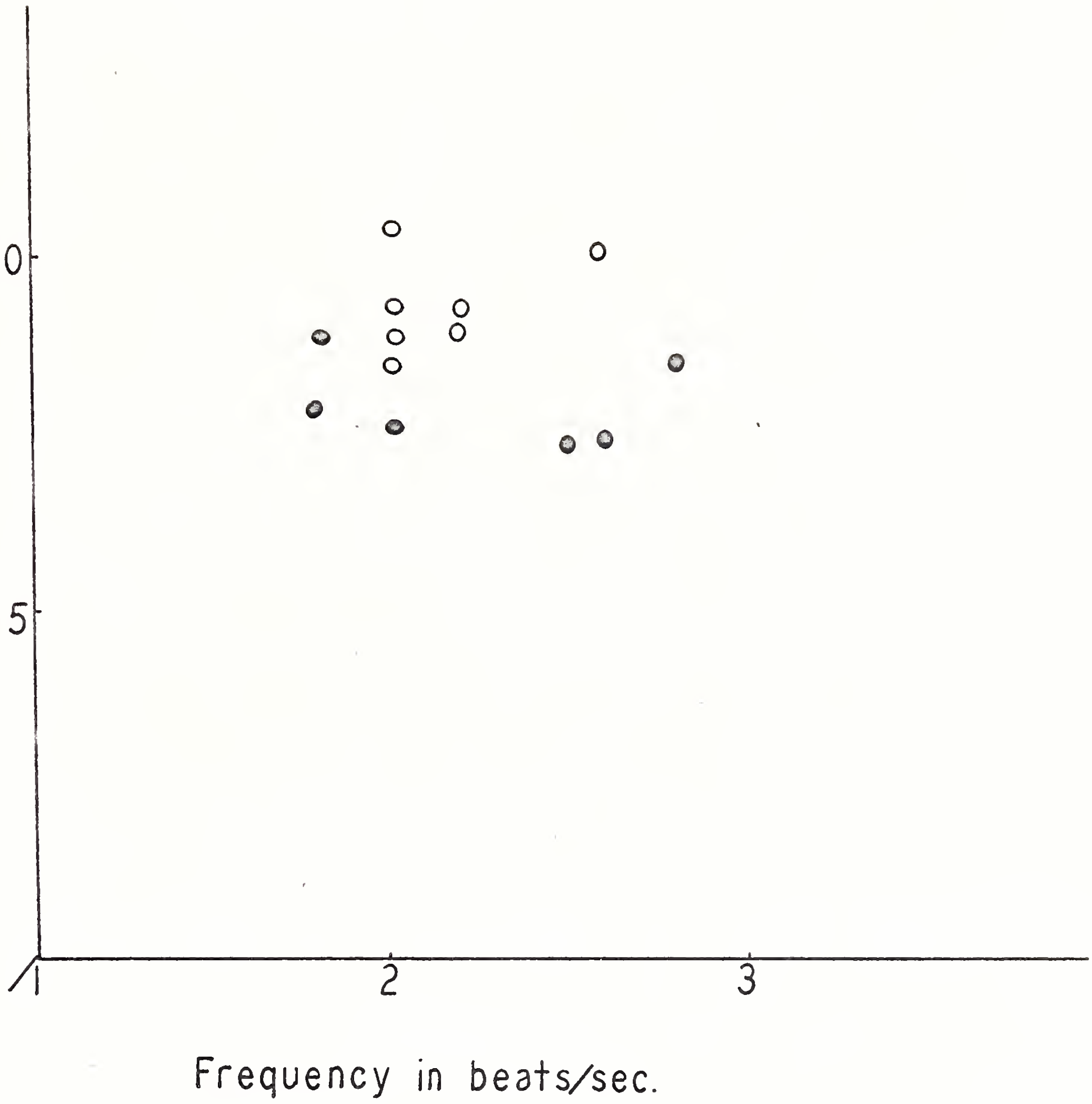


Figure 6

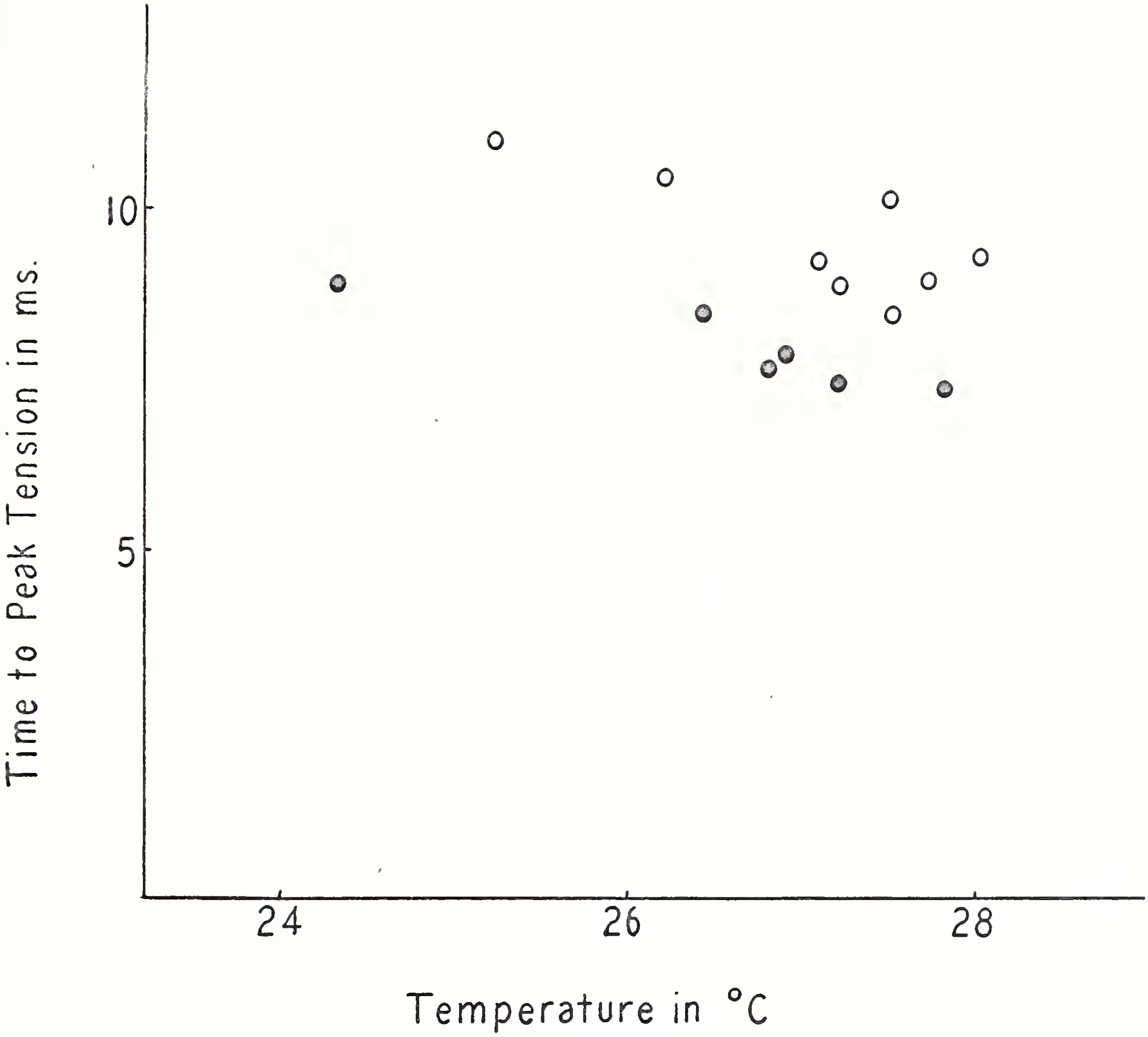


Figure 7

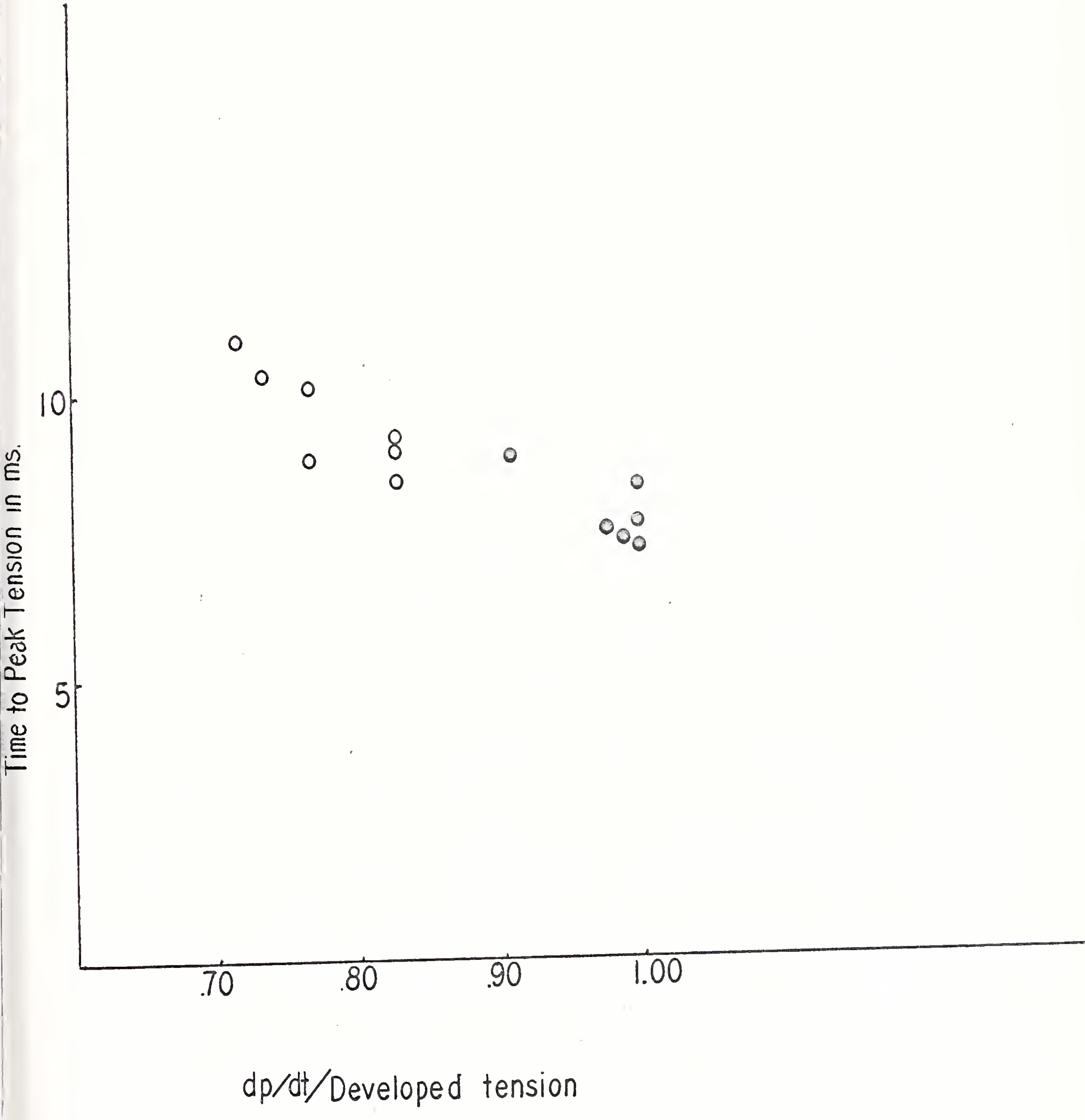

Time to Peak Tension in ms.
10
5
.70
.80
.90
1.00
dp/dt/Developed tension

Figure 8

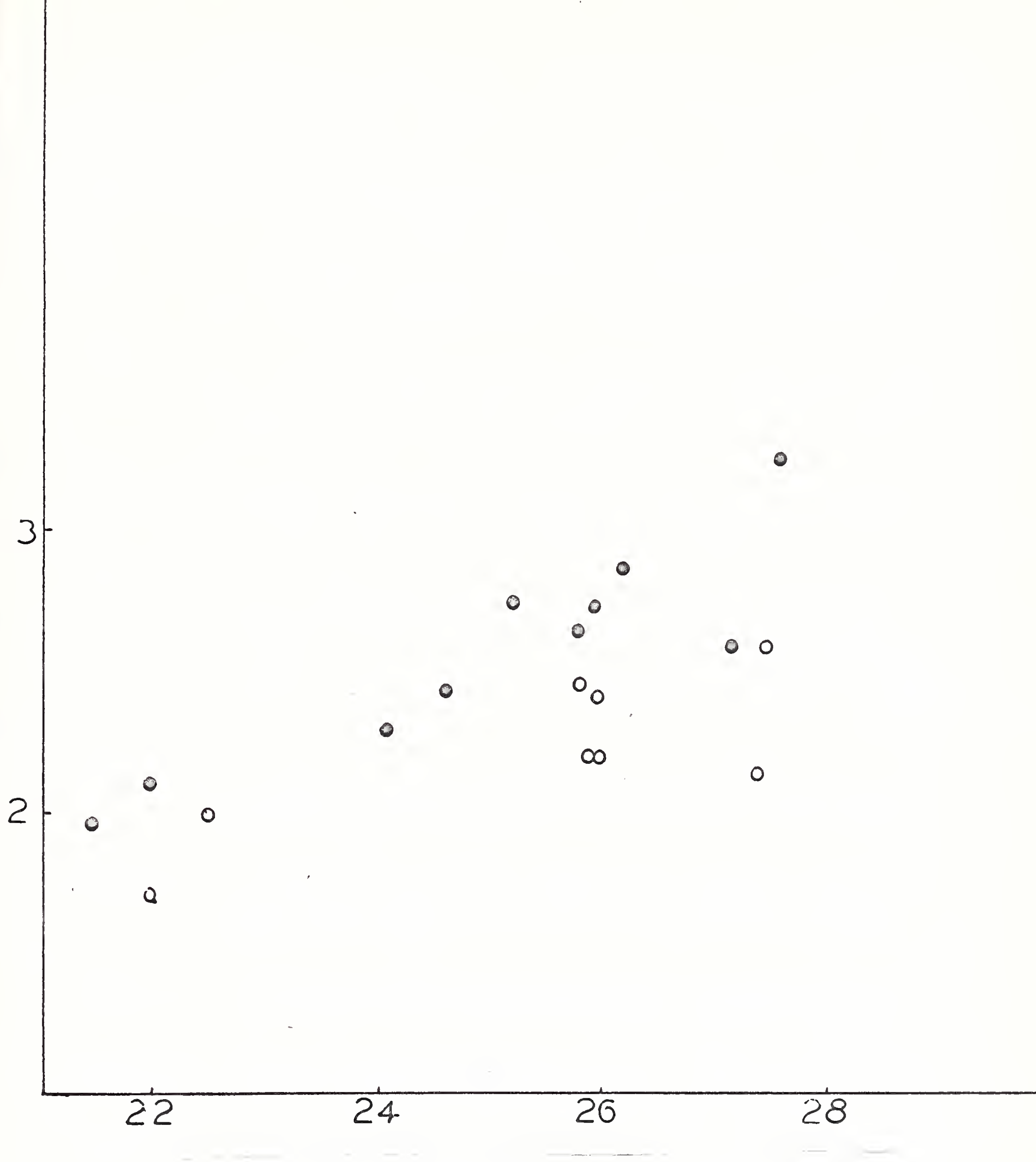


Figure 9

LEGEND

Figure 1: Relationship of the average tension developed to the average percentage change in length in left atria for the group of hyperthyroid animals vs. the group of controls.

Figures 2-9 also represent values obtained from left atria, and symbols seen in these figures are the same as those in Figure I.

Figure 2: Relationship of average frequency of stimulation to the developed tension at an average percent change in length of 50% for a group of normal animals.

Figure 3: Relationship of the average tension developed to the average ratio of dp/dt//developed tension for the group of hyperthyroid animals vs. the group of controls.

Figure 3a: Recording strips from left atria of hyperthyroid and normal animals showing similar developed tensions but demonstrating the difference in the rate of development of tension. Both atria were at the same bath temperature and were stimulated at the same frequency. Sensitivity settings were also the same.

Figure 4: Plot of the ratio of dp/dt//developed tension vs. the frequency at which this ratio was obtained in each atrium for both groups of animals.

Figure 5: Plot of the ratio of dp/dt//developed tension vs. the bath temperature at which this ratio was found for both groups of animals.

Figure 6: Plot of the time to peak tension vs. the frequency at which this time was obtained for both groups of animals.

Figure 7: Plot of the time to peak tension vs. the temperature at which this time was found for both groups of animals.

Figure 8: Relationship of the time to peak tension to the ratio of dp/dt//developed tension for both groups of animals.

Figure 9: Plot of the average frequency of stimulation giving maximal tensions vs. the bath temperature at which this frequency was obtained for both groups of animals.

DISCUSSION

The present experiments have demonstrated a significant increase in the maximum rate of use of developed tension (dp/dt) in the myocardium of hyperthyroid animals as compared to normals under similar conditions. In accordance with the definition of myocardial contractility presented, this increase represented an increase in the degree of activation and thus an increase in myocardial contractility. Koch-Weser and Blinks noted, however, that this increase in dp/dt would increase contractility unless offset by a decrease in the duration of the active state (12). The present study showed the increase in dp/dt to be associated with a small but significant decrease in time to peak tension, the latter signifying a decrease in the duration of the active state. Since the resting tension of each atrium at each particular length setting was not measured or recorded, a direct comparison of developed tension between two atria under comparable conditions was not possible. Thus, whether the decrease in time to peak tension caused a sufficient decrease in peak tension developed to offset the marked increase in dp/dt could not be determined from the data obtained. The data suggest, however, that this effect was not significant. No comparison of the present results with others was possible since none of the published experiment

work on myocardial contractility in hyperthyroid animals measured dp/dt or the time to peak tension.

No significant difference in the maximum tension developed per unit weight of muscle (Tmax/mg muscle) was observed between the two groups of animals. Since resting tensions were not measured in this study, no comparison of Tmax/mg muscle between atria of the two groups at similar resting tensions was possible. Yet comparisons of these values at similar initial lengths indicated no significant difference. Thus, the present results are not in agreement with Whitehorn and Ullrick's report that hyperthyroid ventricular myocardium developed significantly less tension per milligram of muscle than controls at all initial lengths (4). The finding of no significant increase in Tmax/mg muscle in the hyperthyroid animals did not contradict the increase in myocardial contractility in this group as evidenced by the increase in dp/dt, since changes in myocardial contractility can occur without changes in peak tension developed (12).

The finding in the present study that hyperthyroid animals have a higher optimum contractile frequency than normals is in agreement with the findings of Benforado (3). He also found,

however, that at the same resting tension and frequency, hyperthyroid animals developed less tension than controls over the entire frequency range employed. Although resting tensions were not measured directly in the present study, comparisons of tensions developed at similar percentage changes in length indicated no significant difference between the two groups of animals. In reference to this finding, it was noted in Figure I of the Results that the average tension developed per percentage change in length was less for hyperthyroid animals than for controls. This difference was not significant, however, at any percentage change in length. There are two possible causes for this difference. First, normal control heart muscle beat at the average optimal frequency for this group, while hyperthyroid myocardium beat at a frequency less than its average optimal frequency. Thus, control myocardium was contracting to a maximal extent while hyperthyroid myocardium was contracting at a degree less than maximal. Second, high frequencies of contraction maintained artificially during cooling prevents the prolongation of the active state and may result in decreased strength of contraction (12). Since the average bath temperature for hyperthyroid myocardium was 1 degree Centigrade less than for normals while average frequencies of stimulation were the

same, a decrease in the strength of contraction of the hyperthyroid group might have occurred on this basis. In light of the first explanation, Benforado's finding of a significant difference in tensions developed between hyperthyroid and normal animals at similar frequencies could perhaps be explained by the difference in optimal frequencies between the two groups. In a more general sense, the finding of a decrease in myocardial contractility in Benforado's studies did not agree with the finding of an increase in contractility in hyperthyroid animals as compared to normals in the present study. These discrepant findings were notwithstanding the two differing criteria used to substantiate the conclusions.

Brewster *et al.* found that hyperthyroid ventricles *in vivo* developed a lower average contractile force and hypothyroid ventricles a higher average contractile force than normals. This may best be interpreted in light of the spontaneous frequencies at which these forces were measured. In their study, hypothyroid ventricles beat at the lowest frequencies and hyperthyroid ventricles beat at the highest frequencies employed. In a study by Koch-Weser and Blinks, however, of the interval-strength relationships for mammalian ventricles *in vitro*, it was found that a decrease in the interval between beats (and therefore an increase

in frequency) was associated with a steady increase in the strength of contraction (31). The fact that, in the study by Brewster's group, hyperthyroid myocardium beating at a higher frequency developed less tension than hypothyroid myocardium beating at a lower frequency indicates a very significant decrease in myocardial contractility must have occurred in the hyperthyroid group. Brewster *et al.* stated this decrease in contractility was due to the shorter duration of the contracted state, i. e. shorter duration of the active state, in the hyperthyroid ventricle, which in turn was due to the increased metabolic rate in this group. Koch-Weser and Blinks indicated, however, that although an increase in frequency of ventricular muscle is associated with a decrease in the duration of the active state, the increase in the degree of activation with increasing frequency more than offsets this so that strength of contraction increases. Thus, the reason is unclear why this increase in the degree of activation, which would be reflected in the dp/dt or peak tension developed, was not observed in the study by Brewster *et al.* On the contrary, his group found little significant change in the 3 groups of animals in the rate of rise of initial tension at any given temperature. In general, Brewster *et al.* did not clearly differentiate the effects of metabolic rate from those of spontaneous frequency

in myocardial contractility.

Finally, the finding in the present study of lack of a significant difference in the NE content of the myocardium between the two groups was not unexpected. Previous studies had shown that this difference was observed only in extremely thyrotoxic animals (17, 21, 22). Hyperthyroid animals in the present study lost an average of 2.2 grams/day for 13 days treatment with thyroxan, far below extremely thyrotoxic standards.

The lack of correlation between myocardial NE content and contractility in the present study had 3 possible explanations. First, the method used to measure NE content might have been too insensitive to detect small changes in content. Second, if thyroid hormone effected changes in myocardial NE stores by altering the rate of turnover of NE, only extreme changes in turnover rate seen in the markedly thyrotoxic animal would be detected as an increase in NE stores by the methods used (17). Finally, loss of NE into the muscle bath may have played a role in the lack of significant difference in NE content in the two groups. The local release of autonomic transmitter substances as the result of electrical stimulation of heart muscle is known to occur (12). In addition, a high spontaneous release of NE

from its myocardial stores into the muscle bath with the passage of time has been observed (32). In support of both these phenomena in the present study was the finding that right atria showed consistently higher NE content than the left atria, and were also in the muscle bath for a significantly shorter period of time. (Average time in bath for right atria = 48 minutes. Average time for left atria = 96 minutes). The other explanation is that the right atrium contains more NE stores than the left atrium, since the right atrium is known to have a greater sympathetic innervation than the left and endogenous stores of NE are known to be associated with these nerve endings.

The effect of the release of autonomic transmitter substances with electrical stimulation on myocardial contractility is thought to be minimal with the use of small electrodes and stimuli of barely supra-threshold intensity. Marked effects may be produced if mass electrodes and high-intensity stimuli are used (12). The question arises, therefore, whether an indetectable release of NE from stores within the myocardium was not responsible for the increase in contractility seen in hyperthyroid myocardium in the present study. Since guinea pig myocardium is extremely sensitive to very dilute solutions of NE (33), the general effect

APPENDIX

I. Composition of Chenowith-Koelle solution.

NaCl	7.0	gm/liter
KCl	0.42	gm/liter
CaCl $2H_2O$	0.32	gm/liter
Mg Cl_2	0.43	gm/liter
Glucose	1.8	gm/liter
$NaHCO_3$	2.1	gm/liter

Gas Phase: 95% O_2 - 5% CO_2

In making the solution every ingredient except the $NaHCO_3$ was dissolved in distilled water before the gas mixture was bubbled through. The $NaHCO_3$ was dissolved separately and this solution added while the gas mixture was provided not only to the muscle bath, but also to the C-K resevoir for the entire experimental period to prevent the precipitation of $CaCO_3$.

II. Sample method of matching atria from each group according to comparable frequencies and bath temperatures

dp/dt/Developed tension for left Atria

NORMALS				HYPERTHYROID			
GP#	dp/dt/Tension	Frequency of Stimulation beats/sec	Bath Temperature	GP#	dp/dt/Tension	Frequency of Stimulation	Bath Temperature
1	.72	2.0	25.0	8	1.00	2.0	24.6
2	.72	2.0	25.5	9	.85	2.0	25.4
3	.77	2.6	27.5	10	.99	2.6	27.2
4	.83	2.2	26.9	11	.99	2.2	26.8
5	.90	2.2	27.5	12	.93	2.2	26.8
6	.85	2.8	26.2	13	.95	2.8	26.8
7	.83	2.2	27.5	14	1.00	2.5	27.6
Average	.80	2.28	26.6		.96	2.33	26.4

SUMMARY

Isometric contractility of atrial myocardium was studied in 12 hyperthyroid and 15 normal guinea pigs. The maximal rate of rise of developed tension (dp/dt) was significantly greater and time to peak tension significantly lower in the hyperthyroid myocardium as compared to controls under similar experimental conditions. The data thus suggest that myocardial contractility was increased in the hyperthyroid group. This finding was compatible with the known increase in cardiac output and cardiac hypertrophy seen in hyperthyroid animals, but contradicted previous reports of a decreased myocardial contractility in the hyperthyroid animal.

No correlation was found between myocardial contractility and the NE content of the myocardium. The significance of this finding was discussed.

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